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THE FERTILIZATION REACTION IN ECHINARACHNIUS PARMA.

III. THE NATURE OF THE ACTIVATION OF THE EGG BY BUTYRIC ACID.

E. E. JUST.

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I. INTRODUCTION.

The egg of *Echinarachnius* as shown elsewhere (Just, '19a) following engulfment of one sperm immediately becomes immune to farther sperm entry. This change takes place in the cortex *before* the membrane lifts off and probably represents the neutralization of the fertilizin by the anti-fertilizin present in the egg. This is the mechanism for the prevention of polyspermy, and not the immediately succeeding membrane lifting. Loeb, however, found that butyric acid, though it be capable of calling out "fertilization membranes" in one hundred per cent. of the eggs of the sea-urchin, is none the less incomplete in its action, for if by shaking he removes these "fertilization membranes" sperm penetrate the eggs, membranes form anew, and development proceeds like the normal. This finding certainly does not support his view that fertilization is a kind of artificial parthenogenesis for after fertilization eggs or fragments thereof do not

"refertilize" (see older work of Driesch and the important work of Wilson on *Cerebratulus*). The present paper reports results of an attempt to determine whether or not in *Echinarachnius* eggs activation by butyric acid is complete in the sense that it is complete when membranes form after the initial stage in the fertilization process. If we assume that activation is complete in eggs that have formed membranes after butyric acid then hypertonic sea-water exposure should give the best development with these eggs. Any other assumption is absurd unless the use of butyric acid is unnecessary, when used with hypertonic sea-water, for the best type of development. Again, on the assumption that with membrane formation following butyric acid the activation is complete, as it is in fertilization, the egg should react on insemination as do fertilized eggs or fragments thereof. The observations show that after butyric acid treatment under optimum conditions eggs form membranes (Part II.); and that in such eggs activation is complete since with hypertonic treatment they give rise to cleavages, gastrulæ and plutei of a high degree of normality, and since they fail to develop after insemination (Part III.).

II. ACTIVATION OF ECHINARACHNIUS EGGS BY BUTYRIC ACID.

Eggs of *Echinarachnius* if properly exposed to butyric acid in sea-water are activated and so form membranes that are indistinguishable from those formed following insemination.

A. *Optimum Exposure to Butyric Acid.*

Without the experiments being cited it may be said that by using various mixtures of butyric acid in sea-water the best mixture was found to be 2 c.c. of $n/10$ butyric acid plus 50 c.c. of sea-water. The optimum exposure for this mixture discovered by removing samples of eggs from the butyric mixture at five second intervals, is thirty-five seconds. In some cases eggs were exposed for sixteen minutes.

The following experiment of July 8 is cited as typical: 9:50 A.M., 15 dishes each with 250 c.c. of sea-water. 10:18 A.M., 2 c.c. $n/10$ butyric acid plus 50 c.c. of sea-water thoroughly

mixed. 10:20 A.M., shed eggs from one very fine female exposed to butyric mixture, samples being removed at intervals to dishes of 250 c.c. of normal sea-water. Table I. gives the data.

TABLE I.

No.	Length of Exposure.	Per Cent. of "Full Membranes."
1	20 seconds	14
2	25 "	72
3	30 "	85
4	35 "	93
5	40 "	87
6	45 "	83
7	2 minutes	0
8	5 "	0
9	6 "	0
10	7 "	0
11	8 "	0
12	9 "	0
13	10 "	0
14	11 "	0
15	12 "	0

Eggs of nos. 7-15 showed a tendency to go bad; this increased with the length of exposure, for no. 15 eggs were very bad.

The results are essentially the same in the experiments of July 11 and July 26 as shown in Table II.

TABLE II.

No.	Length of Exposure.	Per Cent. of "Full Membranes."	
		July 11.	July 26.
1	20 seconds.	3	18
2	25 "	39	36
3	30 "	50	76
4	40 "	96	91
5	45 "	67	83
6	50 "	49	— ¹
7	55 "	12	— ¹
8	60 "	1	1
9	65 "	0	— ¹

¹ No exposure for this length of time.

During August 4, seven lots of eggs from different females were exposed to 2 c.c. *n*/10 butyric acid plus 50 c.c. sea-water for 20, 25, 30, 35, 40, 60, 90 and 120 seconds. The 35-second exposure in each case gave 91, 90, 98, 93, 99, 97 and 97 per cent. full membranes. Observations of August 6 were about the same.

These citations are sufficient, I think, to show that there is a very definite optimum exposure between rather narrow limits for the activation of *Echinarachnius* eggs by the mixture of butyric acid and sea-water employed. But if the eggs are exposed to *higher* concentrations of butyric acid even for a shorter time they do not form full rounded membranes that stand off from the cortex but thick membranes adhering closely to the cytoplasm. Thus, on July 6, eggs exposed to 3 c.c. of $n/10$ butyric acid plus 50 c.c. of sea-water and those exposed to 3.5 c.c. of the acid plus 50 c.c. of sea-water for 20 seconds each gave one hundred per cent. of these thick contracted membranes. These membranes are entirely different from the so-called "fertilization membranes" got with lower concentrations of butyric used. Apparently, Loeb obtained the same results with *Arbacia* eggs. Speaking of the effect of butyric on this egg he says: "When transferred to sea-water, they did not form a conspicuous fertilization membrane as did the eggs of *S. purpuratus* under the same circumstances, but only a fine gelatinous layer which was not easily visible" (page 71).

On shaking some time after removal from butyric sea-water full membranes partially collapse; contracted membranes on shaking break and the eggs give off buds. These buds show extreme variations in size. Butyric treatment alone (at least for the longest exposure tried, sixteen minutes) never gives cleavage. The eggs never go beyond the monaster stage.

B. *Condition of the Eggs as a Factor in the Response to Activation by Butyric Acid.*

Without exception my best results with *Echinarachnius* eggs have been obtained with dry shed eggs—i. e., eggs deposited by the animals in clean dry watch glasses. It is not always practicable to wait until the animals shed; I, therefore, proceed as follows: The animals are washed in sea-water then in running tap-water, shaken dry and placed aboral side down after a slight cut around the peristome which usually suffices to induce shedding;¹ such eggs are scarcely inferior to normally shed eggs. In

¹ Great care was exercised to keep everything sterile. On only one occasion was an egg found in the two cell stage in the control—not more certainly than one in three or four hundred eggs, but the experiment was discarded.

the experiments cited above the butyric mixture previously prepared was added directly to the Syracuse glass containing the eggs. I was content to work with relatively a small number of eggs always from but one female.¹ The amount of butyric mixture used, therefore, could be likewise small (in my experiments never more than 12.5 c.c.) so that the eggs could be thoroughly stirred up and still settle quickly with the result that when at most the 1 to 1.5 c.c. was removed the amount of acid carried over was negligible.²

1. *Effect of Washing*.—Dry eggs may be washed quickly once or twice in a small amount of sea-water without affecting the butyric treatment. Additional washings will cut down the per cent. of butyric membranes and washings extending over one to two hours will result in the failure of butyric acid to form membranes. Eggs similarly treated may be still capable of fertilization. Eggs incapable of fertilization never respond to butyric acid treatment.

An experiment of July 26 shows the difference between dry and washed eggs:

8:55 A.M. A fine lot of eggs divided among three dishes—A, B and C. Lot A dry; Lot B washed three times in fifty minutes; to lot C two drops of body fluid added.

10.00 A.M. Lot B gave the following results after exposure to butyric sea-water:

Exposure in seconds.....	20	25	30	35	40	60
Per cent. of membranes.....	6	19	38	27	14	—1
Lot A (unwashed) per cent. of membranes.....	18	36	76	91	83	1

On August 4 similar results were obtained. After four washings in thirty minutes the per cent. of membranes formed was reduced one half after optimum exposure as compared with the unwashed.

¹ Indeed, in my judgment the practise of using large quantities of eggs from a number of females is a most pernicious one, introducing as it does more unknown factors. Nothing is so variable as the echinid egg, as Tennent, Medes and Goldfarb have shown—the work of the last-named being particularly concerned with our problem; in a lot of eggs from several females one must get a very heterogeneous population—especially, as is usually the case, if the worker obtains his eggs by mincing the ovaries in sea-water.

² Transfers were sometimes made to 4,000 c.c. of normal sea-water; the results obtained were no better than those to 250 c.c. of sea-water.

Against these observations the objection might be raised that the failure of washed eggs to form membranes in greater number is simply due to the low concentration of butyric acid in sea-water since from the washed eggs all the water cannot be removed. Against this we have (1) the fact that dry eggs quickly washed once or twice may give one hundred per cent. membranes. If, by chance, body fluid was present with shed eggs they were always washed. (2) After washing all but a negligible amount of water can be removed. (3) Diminution in concentration of acid does not explain absolute loss by the egg of the power to respond to butyric acid which may take place in an hour. (4) Finally, washed eggs subjected to butyric acid in higher concentration (*e. g.*, 3 c.c. $n/10$ butyric plus 50 c.c. of sea-water) if they form membranes at all form the thick closely adherent membranes which result from exposure to too great concentration. Washing alone is responsible for the failure of the eggs to form membranes; repeated washings in 10 to 15 c.c. of sea-water for one to two hours or a single washing in 250 c.c.¹ will render eggs incapable of activation by butyric.²

2. *Effect of Body Fluid*.—My 1914 experiments on the initiation of development of *Nereis* eggs through increased temperature proved that serum as definitely inhibits initiation of development, as it does fertilization (Just, '15). I was, therefore, led to study the effects of *Echinarachnius* body fluid on butyric acid activation of *Echinarachnius* eggs. Eggs mixed with body fluid form few if any full membranes; they usually form the thick closely adherent type of membrane.

(a) Thus, the eggs of Lot C of July 26 mentioned on page 43 (eggs plus two drops of body juice) gave the following:

Exposure in seconds.....	20	25	30	35	40	60
Per cent. of membranes.....	0	3	14	16	11	0

(b) July 15, 10:30 A.M. Eggs plus body fluid (2 drops each) in butyric acid sea-water for 20, 25, 30, 35 and 40 seconds.

10:50 A.M. *Not one full membrane*; some eggs have the thick contracted membranes.

¹ I did not wash in the larger quantities often, for with them the difficulty of recovering the small number of eggs increases; moreover, concentrating the eggs takes time—thus introducing another factor.

² Washing may not have the same effect on *Arbacia* eggs which are much more resistant. (See Just, '19b.)

(c) July 8. Eggs shaken from ovaries into butyric mixture gave the following results:

Exposure in seconds.....	20	25	30	35	40	45	50	55
Per cent. of membranes.....	33	38	34	48	31	20	17	0

All contracted membranes.

(d) Experiments of August 4 and 6. Serum eggs form no membranes after butyric acid exposure.

In (d) the cortical changes that are responsible for membrane formation were probably complete (see beyond). The actual lifting off of the membrane is not a *sine qua non* of activation. (See Just, '19b. Loeb, '15, discusses this point apropos certain observations of Brachet.)

Heilbrunn found that in *Arbacia* eggs membrane elevation by acid is inhibited by *Arbacia* blood.

C. Summary of Part II.

Summarizing we find that butyric acid in sea-water activates *Echinarachnius* eggs if they are in best physiological condition; slight washing will not impair their capacity to respond to the acid but long washing will. The body fluid inhibits membrane elevation.

III. ANALYSIS OF BUTYRIC ACID ACTIVATION.

If membrane elevation is a sign of complete activation those eggs exposed for optimum length of time to sea-water with butyric acid in optimum concentration should respond best to subsequent hypertonic treatment. Moreover, activation by butyric if complete as in fertilization should inhibit fertilization. The present section deals with the analysis of butyric acid activation and contains the evidence that shows that such activation is complete.

A. Effect of Hypertonic Sea-water on Butyric Treated Eggs.

Butyric acid acting along on *Echinarachnius* eggs (at least for exposures employed which were up to 16 minutes) never induces cleavage. But after butyric activation hypertonic sea-water causes cleavage—62 per cent. in one case—which is scarcely to be distinguished from the normal; such eggs form gastrulae and

develop into plutei that swim to the top of the dishes. Mixtures in the proportions 5, 6, 7 and 8 c.c. of 2.5 M NaCl plus 50 c.c. of sea-water in each case were employed. The best results were obtained with the mixture of salt and sea-water in the proportions of 5 cc. of 2.5 M. NaCl plus 50 c.c. of sea-water.¹ The procedure was as follows: Eggs exposed to butyric acid sea-water were removed at intervals of 5 seconds to 250 c.c. of sea-water. Following about 20 minutes in sea-water eggs were carried over to dishes of hypertonic sea-water at 15, 20, 25, etc., minutes. Twenty-five minutes treatment is best. *Eggs previously exposed to butyric acid for 35 seconds (optimum exposure for membranes) gave the highest per cent. of cleavage and the best type of development.*

It would be tedious to cite individual experiments on this point. The reader would scarcely be interested in experimental details on the rather threadbare subject of the effect of hypertonic sea-water following butyric activation. It is, of course, generally understood that hypertonic sea-water is most successful subsequent to butyric acid treatment where membranes have been formed. There would certainly be no reason to use butyric acid if this were not true. Notwithstanding all this, I wish to emphasize that those eggs with the highest per cent. membranes (optimum exposure) yield best to hypertonic treatment. Eggs under- or over-exposed to butyric acid do not give the same type of development.²

B. *Effect of Insemination Following Butyric Acid Activation.*

After removal to normal sea-water following optimum exposure to butyric acid sea-water *Echinarachnius* eggs form 100 per cent.

¹ The 2.5 M. NaCl solution was always freshly made. No solution was ever older than six hours when added to the sea-water.

² The successfully treated eggs are certainly very beautiful; as far as the gastrula stage in most cases they appear to be perfectly normal. The plutei develop more slowly than those from fertilized eggs and are not so hardy. Possibly, with even nicer treatment, the development could be improved.

Cytological study of eggs induced to develop with artificial means has been made by Wilson, Hindle and Herlant. In the living *Echinarachnius* egg we observe that while in sea-water following butyric acid treatment a monaster forms beyond which stage the egg does not develop. Having been properly exposed to hypertonic sea-water the egg forms in sea-water a complete astral system. Overexposure to hypertonic sea-water results in the formation of innumerable asters when the egg is removed to sea-water: such eggs fail to cleave or form very irregular cleavages.

membranes in highly successful cases. Such eggs mixed with sperm do not develop though the membranes be previously removed as soon as formed. Thus, activation with butyric acid is complete; eggs with membranes have undergone an irreversible change.

The Experiments.—The method followed in these experiments is simple. Eggs are exposed to $n/10$ butyric acid in sea-water (2 c.c. of acid plus 50 c.c. sea-water) and removed to 250 c.c. of normal sea-water at five second intervals; these dishes comprise Series *A*. From the dishes of series *A* eggs are removed, shaken as soon as membranes are formed, and inseminated with fresh sperm suspension; thus, series *B* is established. Samples of eggs from each of the *A* dishes inseminated without shaking off the membranes make up series *C*. The membranes formed in series *A* and later the cleavages in *B* and *C* are recorded; *B* and *C* dishes are then gently washed in fresh sea-water and set aside for study of the development during the next twenty-four hours.

The experiment of July 9 is typical: 10.00 A.M. 6 dishes each with 250 c.c. of sea-water to which eggs are removed after exposure to butyric sea-water after 30, 35, 40, 60, 120 and 180 seconds (series *A1* to *A6*). Samples from *A1* to *A6* shaken and inseminated (series *B*, 1 to 6). Series *C* samples from *A1* to *A6* inseminated without shaking. Table III. gives the results:

TABLE III.

No.	Exposure in Seconds.	Per Cent. of Membranes in <i>A</i> .	Per Cent. of Cleavages,		Per Cent. of Swimmers.	
			In <i>B</i> .	In <i>C</i> .	In <i>B</i> .	In <i>C</i> .
1	30	70	27	28	7	6
2	35	76	30	27	6	3
3	40	74	29	23	0	1
4	60	5	13	3	2	4
5	120	0	30	43	44	41
6	180	0	48	44	41	39

B4 to *B6* and *C4* to *C6*: cleavages very irregular; "swimmers" extremely abnormal—exogastrulæ, microgastrulæ, blastulæ with dense opaque interiors evidently due to abortive gastrulation.

I have given this particular experiment because not a very high per cent. of the eggs formed membranes. This ought, therefore to be crucial, for since under-exposed eggs (*i. e.*, those which are

do not form membranes after short exposures) may fertilize perfectly we may assume that these particular eggs were on the border line of proper exposure. Insemination, however, caused no membranes (cf. Herbst) in the over-exposed eggs, and this is always the case, though some of the eggs cleave.

Membranes once formed may be removed, but after insemination these eggs develop no farther than uninseminated butyric treated eggs. Study of the figures given in Table III. shows first, that the cleavages of unshaken inseminated eggs (with membranes) and of shaken inseminated eggs (without membranes) are approximately equal; and second, that the per cent. of membranes in *A* plus the per cent. of cleavages in *B* or *C* is very close to a hundred. This must mean that eggs with membranes formed with butyric acid are not fertilizable. In a given lot of eggs exposed for the optimum time to butyric some are not completely activated and have not formed membranes. These are the eggs that cleave following insemination; the removal of the membranes does not affect the per cent. of cleavages thus obtained.

The experiment of July 8 part of which has already been given (p. 40) is clearly decisive. Following exposure to butyric sea-water Series *A* eggs were shaken and inseminated (series *B*) with the results found in Table IV.

TABLE IV.

No.	Exposure.	Per Cent. of Membranes (Series A).	Per Cent. of Cleavage After Insemination (Series B).
1	20 seconds.	14	50
2	25 "	72	26
3	30 "	85	15
4	35 "	93	6
5	40 "	87	16
6	45 "	83	19
7	2 minutes	0	41
8	5 "	0	43
9	6 "	0	—
10	7 "	0	—
11	8 "	0	—
12	9 "	0	—
13	10 "	0	—
14	11 "	0	—
15	12 "	0	—

B7 to B15: cleavages very abnormal; frequently incomplete many spindles present.

July 26, August 4 and August 6. Eggs after 35 seconds exposure showed 100 per cent. membranes but failed to develop following insemination whether freed of membranes or not.

Early in the season eggs were found that failed to form a single membrane subsequent to optimum butyric treatment. Such eggs on insemination never developed. Eggs inhibited by the body fluid may not form membranes; if they be inseminated they do not form membranes or otherwise develop. Such eggs have undergone cortical changes but membrane formation is suppressed. In serum eggs the suppression usually is not complete: the membrane thickens and remains stuck to the egg.

Moore working with *Arbacia* found that those eggs that form butyric membranes do not develop after insemination though the sperm may penetrate. Whether under similar conditions sperm enter the eggs of *Echinarachnius* I am unable to say. Mere penetration, however, in no wise affects our position. Development does not depend on sperm penetration simply; development begins with certain cortical changes which having been initiated by agents other than the sperm inhibit action by superimposed sperm.

C. Summary of Part III.

We may summarize the results of this section as follows: Butyric acid eggs with membranes respond best to hypertonic sea-water giving development that closely simulates the normal. Eggs with butyric membranes cannot be fertilized though the membranes be removed. These facts indicate that cortical activation is complete.

IV. DISCUSSION.

1. Workers generally appreciate how capricious is the response of eggs to agents that initiate development. Often the procedure of a given experiment may be repeated with altogether different results. Hence we conclude that the varying "physiological condition" of the eggs is responsible for our results. Study of fertilization in *Echinarachnius* (Just, '196) shows that this physiological condition depends on the presence of fertilizin: eggs that have a high fertilizin index show a high per cent. of

development; eggs washed free of fertilizin lose their fertilizing power; immature eggs and fertilized eggs show no fertilizin reaction and are incapable of fertilization (and re-fertilization).

Now, development of sea-urchin ova and of marine ova generally by chemical and physical agents is of course purely artificial; it is at least debatable that these agents are as efficient for initiating development as specific sperm. In *Nereis* and in *Echinarachnius* the volume of evidence is incontrovertible: only those eggs in best condition (as shown by their fertilizin tests) respond to artificial agents that initiate development. If eggs from a given lot fail of fertilization the remainder of that lot will not respond to artificial agents. Indeed, in both *Nereis* and *Echinarachnius* the egg through washing—and loss of fertilizin—loses its power to respond to warming (*Nereis*) or to butyric acid (*Echinarachnius*) before it loses its power to respond to sperm. Eggs with full fertilizin content most nearly ensure success with artificial agents.

Blood inhibits fertilization—a truism to the embryologist; where marine ova are normally shed and insemination takes place in the sea, it is generally found best to wash the eggs before insemination. Normally shed eggs always give a high per cent. of development; eggs cut or washed from the ovaries are not so good. Thus, in *Podarke* and in *Cumingia*, for example, eggs develop in greatest number if laid. The blood blocks fertilization “by occupying the ovophile group of the fertilizin, thus preventing action of the latter upon the egg by union with egg receptors” (Lillie). It probably acts in the same way in artificial initiation of development.

In *Nereis* and in *Echinarachnius* blood inhibits both fertilization and artificial initiation of development. In *Arbacia* Lillie found that blood inhibits fertilization while Heilbrunn observed that it also inhibits membrane formation. The worker, therefore, who studies “artificial parthenogenesis” is between Scylla and Charybdis—either he will not wash the eggs and thus have blood inhibitor present, or he will wash too much and so bring about too great secretion of fertilizin. This may seem fanciful to some but experience with *Nereis*, *Platynereis* and *Echinarachnius* teaches that the egg is anything but an inert cell; for suc-

cessful treatment with artificial agents it must be handled with extreme care to insure uniform results.

2. *Echinarachnius* eggs that form membranes through butyric acid treatment are completely activated since such eggs respond to subsequent hypertonic sea-water treatment with development that closely simulates the normal, and since eggs under- or over-exposed to butyric acid and which do not form membranes never form membranes following hypertonic sea-water treatment nor do they develop at all normally. Activation, again, must be regarded as complete since butyric acid eggs with membranes fail to develop after insemination even though the membranes be removed as soon as formed; in this they resemble fertilized eggs or fragments thereof that fail to re-fertilize. Finally, these butyric acid treated eggs with membranes fail to give the fertilizin test as rapidly as do fertilized eggs. We must, therefore, conclude that activation is as complete in eggs with butyric membranes as in eggs with fertilization membranes.

It is interesting to note that Loeb found that his sea-urchin's eggs after butyric acid membrane formation were capable of fertilization provided he had removed the membranes by shaking. Lillie, however, found that *Arbacia* eggs after membrane formation by butyric acid cease to produce fertilizin as do fertilized eggs. Moore confirmed Lillie's butyric observation on *Arbacia* and also discovered that eggs with butyric membranes are incapable of fertilization. To Loeb, complete membrane formation by butyric is an incomplete activation; to the workers with *Arbacia* and *Echinarachnius* eggs membrane formation by butyric acid is a complete activation. In initiation of development, therefore, the egg gives a similar response both to the artificial agent and to the spermatozoa. This, however, does not entail subscription to the famous "lysin theory."

It is now some twenty years since the work on artificial parthenogenesis began. During this time eggs have been subjected to every kind of agent imaginable, a vast amount of literature has been produced, and many guesses have been made on the basis of this work as to the nature of the fertilization reaction. This work on artificial parthenogenesis constitutes one of the important chapters in the history of modern biology and no

mean part among the many brilliant achievements of Loeb which aside from their inherent value have another claim to distinction—their stimulating effect on the new experimental zoölogy. But *artificial parthenogenesis is not fertilization*. And it is indeed unfortunate that the term artificial parthenogenesis ever had that connotation. Thus we read of “fertilization membranes” by this or that chemical, “fertilizing agents,” “chemical fertilization,” etc., when we know that there are eggs that will respond to very slight changes in the environment. The eggs of *Amphitrite*, *Asterias*, *Nereis*, etc., for example, will respond simply to agitation. Artificial parthenogenesis *per se* is too significant to masquerade as fertilization. The processes initiated in artificial parthenogenesis and fertilization probably are the same. But to argue that the sperm carries a lysin because a host of agents activate the egg is bad logic. It is far simpler to postulate that the egg contains the necessary mechanism for development. Such a postulate embraces not only artificial parthenogenesis and fertilization, but also the widely occurring and *real* parthenogenesis. Artificial parthenogenesis teaches us that the egg is a highly irritable cell that will respond to various agents; it has placed us but little nearer a solution of the fertilization problem than we were twenty years ago. One might as easily deduce from the contraction obtained on a curarized muscle with any one of a number of stimuli the nature of the nerve impulse as to formulate a theory that since CO₂, or any other of a number of agents induces development fertilization is due to a lysin carried by the sperm.

Nor yet have purely morphological hypotheses proved satisfactory. The Boveri hypothesis as its predecessors is no longer considered adequate; and such deductions as those of Meves lead only to absurdities.

Lillie's assumption that the egg in its fertilizable condition contains a substance (fertilizin) which needs merely to be activated is logical and is based on observation and experiments which themselves, theory aside, excite admiration. Future work will determine how much this theory has helped toward a solution of the fertilization problem.

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